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Acute Toxicity and Sublethal Behavioral Effects of Copper on Barnacle Nauplii 'Balanus improvisus'

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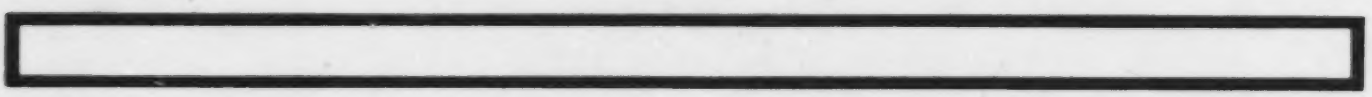

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16. ABSTRACT

This study documents the effects of short-term (24 h) sublethal copper exposures on undirected swimming activity and photobehavior of Balanus improvisus stage II nauplii. All Cu treatments were static, with temperature and salinity conditions at 20°C and 15 or 30°/oo. The 24 h LC 50 estimate for Cu is 88 ppb at 15°/oo and >200 ppb at 30°/oo. Sub-lethal Cu concentrations cause reductions in swimming speed, which decrease progressively with increasing Cu dose. At 50 ppb Cu, this was significant primarily at light intensities below the phototactic threshold. At high Cu concentrations, significant reductions in mean linear velocity occurred at most light intensities tested. At 30°/oo, 50 and 100 ppb Cu also reduce the positive phototactic response and 150 ppb Cu causes reversal of phototaxis at optimal intensities. Photokinesis is reduced at 100 ppb Cu and disappears at 150 ppb Cu. At 15°/oo, the behavioral effects of 50 ppb Cu resemble those occurring with 150 ppb Cu at 30°/oo. Swimming speed and photobehavior show promise as sensitive behavioral indicators of copper toxicity. Additional research is required to determine if these responses apply to a broad range of pollutants and to other planktonic organisms. There is also a need to further evaluate the significance of these behavioral effects ecologically.

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Acute Toxicity and Sublethal Behavioral Effects of Copper on Barnacle Nauplii *(Balanus improvisus)**

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Abstract

This study documents the effects of short-term (24 h) sublethal copper exposures on undirected swimming activity and photobehavior of *Balanus improvisus* stage II nauplii. All Cu treatments were static, with temperature and salinity conditions at 20 °C and 15 or 30 ‰. The 24 h LC 50 estimate for Cu is 88 ppb at 15 ‰ and >200 ppb at 30 ‰. Sub-lethal Cu concentrations cause reductions in swimming speed, which decrease progressively with increasing Cu dose. At 50 ppb Cu, this was significant primarily at light intensities below the phototactic threshold. At higher Cu concentrations, significant reductions in mean linear velocity occurred at most light intensities tested. At 30 ‰, 50 and 100 ppb Cu also reduce the positive phototactic response and 150 ppb Cu causes reversal of phototaxis at optimal intensities. Photokinesis is reduced at 100 ppb Cu and disappears at 150 ppb Cu. At 15 ‰, the behavioral effects of 50 ppb Cu resemble those occurring with 150 ppb Cu at 30 ‰. Swimming speed and photobehavior show promise as sensitive behavioral indicators of copper toxicity. Additional research is required to determine if these responses apply to a broad range of pollutants and to other planktonic organisms. There is also a need to further evaluate the significance of these behavioral effects ecologically.

Introduction

Although copper is an essential trace element in living organisms, it may become toxic at increased concentrations induced by anthropogenic disturbances and perhaps naturally occurring variations (Harrison *et al.*, 1977). The median concentration of Cu in nearshore waters is about 1.5 ppb; values above 3.0 ppb probably reflect unnatural disturbances (Schmidt, 1978b). Estuaries with heavy industrial and municipal pollution may exhibit particularly high Cu concentrations with up to 27 ppb Cu reported from Boston Harbor, USA and 65 ppb Cu reported for Lower New York Bay (Waldhauer *et al.*, 1978). Because of its widespread use, Cu represents a very high potential for pollution in aquatic systems (Bowen, 1966).

The acute toxicity (24 to 96 h LC50) of copper to marine organisms varies considerably with test species and test conditions but reported lethal concentrations are generally above 50 ppb Cu (Black *et al.*, 1976). Although simple and inexpensive short-term acute toxicity tests can provide useful information on the relative toxicity of test substances and relative sensitivity of test organisms, they inherently lack the resolution to detect chronic and sublethal threshold concentrations–information which is essential to determine "safe" concentrations for water quality criteria (Sprague, 1976). In the case of Cu, chronic toxicity studies with freshwater fish have shown "no effect" levels are only 3 to 13% of 96 h LC50 concentrations (Mount, 1968; Mount & Stephan, 1969; McKim & Benoit, 1971). In the marine environment, inhibition of growth has been observed at 5 to 20 ppb Cu for algae (Harrison *et al.*, 1977) and 10 to 13 ppb Cu for hydroids (Stebbing, 1976). In controlled ecosystem experiments, a decline in copepod populations occurred at 10 ppb Cu, but these results could reflect alterations on lower trophic levels rather than a direct effect on the copepods (Gibson & Grice, 1977). Moraitou-Apostolopoulou and Verriopoulos (1979) noted suppressed longevity and fecundity of *Acartia clausi* exposed to similar Cu concentrations.

Ideally, long-term multiple generation chronic tests (e.g. McKim *et al.*, 1976) and controlled environmental pollution experiments (e.g. Menzel, 1977) could provide sufficient toxicity information, but the cost and complexity of these studies limits their use. Short-term toxicity tests with increased sensitivity and predictive

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value are needed. Two approaches to this goal have been to reduce chronic exposure tests to include only "critical life stages" (Macek & Sleight, 1977) and to use sublethal indicators of toxic effects such as growth, fecundity, metabolism, and behavior (Sprague, 1971; Davis, 1976; Eisler, 1979).

Behavioral responses are generally sensitive indicators of stress (Olla, 1974) and represent potentially useful indicators for toxicity tests and monitoring (Eisler, 1979; Miller 1980). The use of video-computer techniques (Wilson and Greaves, 1979) provides a means to quantify and compare movement parameters of test organisms accurately, thus minimizing an often significant technical problem in conducting behavioral studies (Olla, 1974). Amiard (1976) and Forward and Costlow (1976, 1978) have shown that crab zoeae will alter swimming speed and phototaxis in response to sublethal concentrations of metals (Co, Ag) and insect juvenile hormones. These parameters appear to represent sensitive indicators of pollutant effects. This study has undertaken to determine changes in swimming and phototaxis of barnacle nauplii following 24 h exposure to Cu and to compare the threshold of these responses to acute toxicity results.

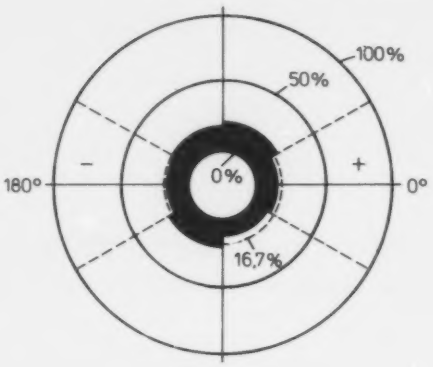

Fig. 1. A key to polar histogram presentations. The percent of a sample group oriented in each of six 30° directional intervals is graphically presented by a polar histogram. A collimated light stimulus is presented at "0°"; positive phototaxis is considered a direction of travel ± 30° of this point, indicated by dashed radial lines; negative phototaxis is considered ± 30° in the opposite direction (180°). Solid circles indicate 100% and 50%; a dashed circle at 16.7% indicates the value for a uniform distribution. Experimental results are based on a mean direction of travel calculated for each nauplii during a given time interval

Materials and Methods

Balanus improvisus nauplii were sorted from plankton samples taken at Pettaquamscutt River near Narragansett, Rhode Island, USA. Stage II nauplii were predominant during collection times and easily sorted from the plankton samples (Lang, 1979; Lang *et al.*, 1979a). Nauplii were initially transferred to filtered seawater at 15 °C, 15 ‰ salinity. The water temperature was then allowed to equilibrate to 20 °C over approximately a 2 h period. Nauplii to be tested at 30 ‰ were then transferred to higher salinity water by 5 ‰ increments at 1 h intervals. All nauplii were maintained at final test conditions (20 °C; 15 or 30 ‰) overnight under constant illumination.

For acute toxicity studies, test groups of approximately 40 nauplii each were placed in Carolina culture dishes with 60 ml of seawater and a series of increasing Cu concentrations. A primary stock of 10,000 ppm Cu in dilute nitric acid was diluted to a 1 to 4 ppm Cu secondary stock solution using deionized water. Final test solutions were obtained by serial dilution with 1 μm filtered seawater (15 or 30 ‰). At final dilutions no change in pH or salinity was evident.

Total copper was analyzed using a Perkin Elmer model 360 atomic absorption spectrophotometer equipped with a HGA 2100 heated graphite furnace. All samples, aside from the controls, were diluted prior to analysis. Absorbance of samples was used to quantify Cu as measured against standards in a similar matrix. No attempt was made to determine the partitioning of Cu chemical states within the system.

At 24 h exposure, living nauplii (movement of appendages) were placed in filtered seawater with a mixed algal diet of *Tetraselmis suecia* and *Isochrysis galbana*. After 24 h a final count of surviving nauplii was determined. Reported mortalities include deaths occurring both during the 24 h exposure and 24 h "recovery" periods.

For the determination of naupliar photokinesis (swimming speed) and phototaxis (swimming direction) in response to a horizontal light stimulus at 480 nm, additional duplicate cultures of stage II nauplii were exposed as described for earlier acute toxicity studies to 6 salinity-copper conditions (Fig. 2,3). A 480 nm light stimulus was used, as stage II nauplii have been found to be most sensitive to this wavelength (Lang *et al.*, 1976a).

Following 24 h exposure, replicate samples of approximately 20 nauplii each were transferred to 1.2 × 1.2 cm lucite chambers for video-taping of photobehavior. Nauplii were light-adapted for at least 1 h using room lights supplemented with a 60 w incandescent bulb (Lang *et al.*, 1979a). Taping was conducted in a 20 °C temperature controlled darkened room. Each sample was placed on a Wild dark-field base with substage illumination filtered to 830 nm (15 nm halfwidth). A Cohu 4400 television camera mounted above the chamber recorded larval movement in a horizontal plane (Lang *et al.*, 1979b).

A light stimulus presented horizontally was provided by a 300 w tungsten bulb projector interference filtered (Ditric Optics, Inc.) to 480 nm (about 7 nm halfwidth). Intensity of the stimulus was regulated with neutral density filters and duration controlled by an electromagnetic shutter. Each sample of nauplii was tested at 7 intensities of 480 nm light presented in ascending

order with 45 sec intervals between each 2.5 sec stimulus (Lang *et al.*, 1979a).

Video tapes of naupliar swimming responses were played back through a video-to-digital processor (Greaves & Wilson, 1979) for computer analysis of mean linear velocity (MLV) and direction of travel (DOT). A detailed account of computer analysis is given in Lang *et al.*, (1979a).

Results are presented as the MLV of nauplii during the entire 2.5 sec light stimulus and the DOT during the latter 2.0 sec of the stimulus (see Lang *et al.*, 1979a). A DOT ± 30° in the direction of the light stimulus is considered positive phototaxis while a DOT ± 30° in the opposite direction is considered negative phototaxis. Polar histograms, described in Fig. 1, graphically portray directional results. Sample DOT distributions were tested using a x^2 test (Batschelet, 1965). Standard SAS (Bar *et al.*, 1976) analysis of variance (GLM) and Student's *t*-test programs were used to test MLV data.

Results

The results of the 24 h static exposure to Cu are presented in Table 1. Within the range of Cu concentra-

Table 1. *Balanus improvisus*. Total copper (atomic absorption) at each exposure level and stage II naupliar mortality. Nauplii were exposed to copper for 24 h without food. At 24 h nauplii were removed to filtered seawater with algae; percent mortality is based on the total number of nauplii dead during both the 24 h exposure period and 24 h "recovery" time. Average mortality ± standard deviation is based on 3 to 6 replicate cultures at each condition

	Cu level	PPb±SD		N	% Mortality 24 h recovery
15 ‰	Control	< 2		123	5.0 ± 2.0
	20	17.4	1.3	116	7.6 ± 9.0
	50	48.7	1.5	118	5.3 ± 2.5
	100	111.3	2.1	115	64.3 ± 4.0
	150	150.0	2.5	120	93.0 ± 3.5
	200	201.0	3.8	127	100 ± 0.0
30 ‰	Control	3.0	2.0	316	3.0 ± 3.2
	20	22.2	2.8	315	3.0 ± 3.7
	50	56.6	5.7	318	2.1 ± 2.0
	100	111.0	9.6	318	4.3 ± 4.8
	150	147.0	6.2	316	21. ± 2.9
	200	201.0	5.2	306	8.6 ± 5.4

Table 2. *Balanus improvisus*. The mean linear velocity (MLV) of stage II (30 ‰, 20 °C) nauplii to a 480 nm light stimulus at different intensities. The sample number (N) is the number of naupliar paths analyzed. Homogeneous subsets (subset) as determined by the Duncan's multiple range test ($P \leq 0.05$) are given for MLV results (see Nie *et al.*, 1976). An asterisk indicates a significant ($P \leq 0.05$) difference from respective control values

	Wm^{-2} stimulus 480 nm	N	$mm\ sec^{-1}$ MLV	Subset
Control	4.5×10^{-1}	36	2.42	A
	$\times 10^{-2}$	31	2.34	A
	$\times 10^{-3}$	31	1.94	B
	$\times 10^{-4}$	39	1.71	B
	$\times 10^{-5}$	33	1.53	B
	$\times 10^{-6}$	47	1.70	B
	$\times 10^{-7}$	28	1.79	B
50 ppb Cu	4.5×10^{-1}	38	1.90	A
	$\times 10^{-2}$	45	1.78	AB
	$\times 10^{-3}$	34	1.79	AB
	$\times 10^{-4}$	48	1.48*	B
	$\times 10^{-5}$	44	1.56	AB
	$\times 10^{-6}$	34	1.42*	B
	$\times 10^{-7}$	44	1.54*	AB
100 ppb Cu	4.5×10^{-1}	38	1.68*	A
	$\times 10^{-2}$	35	1.44*	B
	$\times 10^{-3}$	39	1.38*	B
	$\times 10^{-4}$	49	1.45*	B
	$\times 10^{-5}$	56	1.45	B
	$\times 10^{-6}$	43	1.43*	B
	$\times 10^{-7}$	43	1.43*	B
150 ppb Cu	4.5×10^{-1}	44	1.28*	A
	$\times 10^{-2}$	28	1.39*	A
	$\times 10^{-3}$	35	1.05*	A
	$\times 10^{-4}$	34	1.06*	A
	$\times 10^{-5}$	32	1.11*	A
	$\times 10^{-6}$	34	1.14*	A
	$\times 10^{-7}$	33	1.17*	A

Table 3. *Balanus improvisus*. The mean linear velocity (MLV) of stage II (15 °/₀₀, 20 °C) nauplii to a 480 nm light stimulus at different intensities. The sample number (N) is the number of naupliar paths analyzed. Homogeneous subsets (subset) as determined by the Duncan's multiple range test ($\alpha = 0.05$) are given for MLV results (see Nie *et al.*, 1976). An asterisk indicates a significant ($P \leq 0.05$) difference from respective control values

	Wm^{-2} stimulus 480 nm	N	$mm\ sec^{-1}$ MLV	Subset
Control	4.5×10^{-1}	56	2.26	A
	$\times 10^{-2}$	54	1.93	B
	$\times 10^{-3}$	60	1.81	BC
	$\times 10^{-4}$	53	1.91	B
	$\times 10^{-5}$	54	1.63	BC
	$\times 10^{-6}$	55	1.54	C
	$\times 10^{-7}$	50	1.57	C
50 ppb Cu	4.5×10^{-1}	24	1.75	A
	$\times 10^{-2}$	30	1.77	A
	$\times 10^{-3}$	35	1.44*	ABC
	$\times 10^{-4}$	36	1.62	AB
	$\times 10^{-5}$	29	1.32*	BC
	$\times 10^{-6}$	39	1.22*	C
	$\times 10^{-7}$	31	1.20*	C

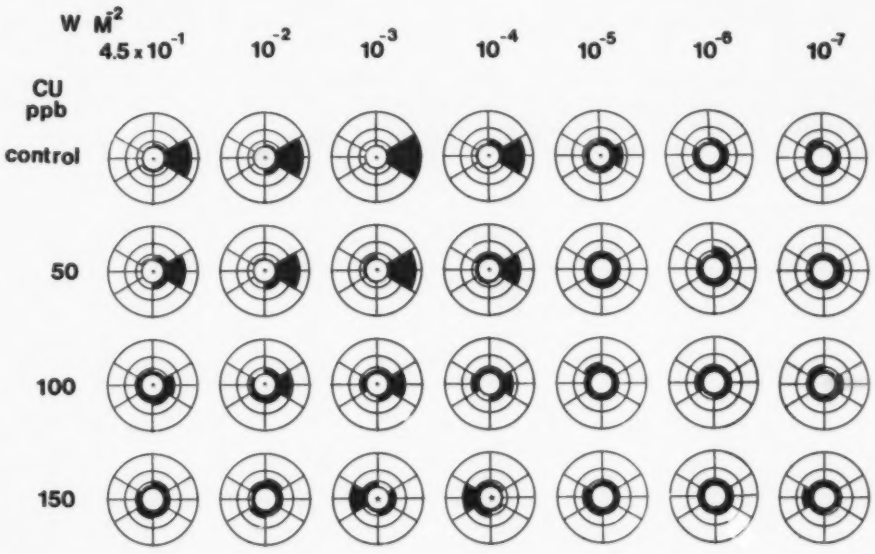

Fig. 2. *Balanus improvisus*. Directional response of stage II nauplii at 30 °/₀₀ salinity after 24 h exposure without food to indicated concentrations of total copper. Polar histograms (see Fig. 1) indicate percent response of mean direction of travel for 28 to 56 nauplii at each condition during the latter 2.0 sec of a 2.5 sec 480 nm light stimulus of indicated intensity. Sample numbers are given in Table 2. A null hypothesis that the distribution of direction of travel means for each sample group is equal to a theoretical uniform distribution was tested using the X^2 test. Significantly ($P \leq 0.05$) nonrandom distributions are indicated by an asterisk

tions tested (0 to 200 ppb), 50% mortality did not occur for nauplii at 30 °/₀₀ salinity. In contrast, nauplii at 15 °/₀₀ salinity exhibited over 50% mortality at 100 ppb Cu and greater. A 24 h LC50 is estimated to be 88 ppb Cu for the 15 °/₀₀ group while at 30 °/₀₀ the 24 h LC50 is greater than 200 ppb Cu.

The MLV and DOT of control and exposed nauplii are presented in Tables, 2,3 and Fig. 2,3. Light-adapted control nauplii have a lower threshold for significant positive phototaxis at about $10^{-6}\,Wm^{-2}$ (Figs. 2,3). The control nauplii display a photokinetic response in that MLV increases significantly ($P \leq 0.05$) in response to higher light intensities at about $10^{-2}\,Wm^{-2}$ and greater (Tables 2,3).

At 30 °/₀₀ salinity, a clear pattern of progressively reduced MLV with increasing Cu concentration is evident (Table 2). MLV at light stimuli below the threshold of phototaxis are significantly reduced at all

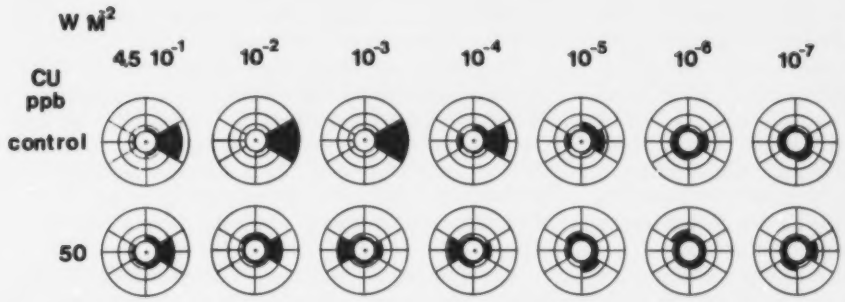

Fig. 3. *Balanus improvisus*. Directional response of stage II nauplii at 15 ‰ salinity after 24 h exposure without food to indicated concentrations of total copper. Polar histograms (see Fig. 1) indicate percent response of mean direction of travel for 24 to 60 naupplii at each condition during the latter 2.0 sec of a 2.5 sec 480 nm light stimulus of indicated intensity. Sample numbers are given in Table 3. Significantly ($P \leq 0.05$) nonrandom distributions are indicated by an asterisk (see Fig. 2)

Table 4. Acute toxicity of copper to marine crustacean zooplankton

Test organism	LC50 ($\mu g\, l^{-1}$ Cu)	Time (h)	Temp. C	Salinity ‰	Reference
Copepods					
Acartia clausi	34–48	48	18 °C	-	Moraitou-Apostolopoulou, 1978
Acartia tonsa	104–311	24	21 °C	36	Reeve *et al.*, 1976
Acartia tonsa	9–78	72	20 °C	30	Sosnowski *et al.*, 1979
Acartia tonsa	17–55	96	20 °C	30	Sosnowski and Gentile, 1978
Calanus plumchrus	2778	24	13 °C	27	Reeve *et al.*, 1976
Labidocera scotti	132	24	21 °C	36	Reeve *et al.*, 1976
Cirripede nauplii					
Balanus crenatus	410	6	-	-	Pyefinch & Mott, 1948
Balanus amphitrite (II)	≅250	24	20 °C	30	Lang *et al.*, 1979b
Balanus improvisus	≅165	24	26 °C	30	Lang *et al.*, 1979b
Balanus improvisus	>200	24	20 °C	30	present study
Balanus improvisus	88	24	20 °C	15	present study
Euphausids					
Euphausia pacifica	14–30	24	13 °C	27	Reeve *et al.*, 1976
Decapod larvae					
Homarus gammarus	100–300	48	15 °C	–	Connor, 1972
Crangon crangon	330	48	15 °C	–	Connor, 1972
Carcinus maenas	600	48	15 °C	–	Connor, 1972
Paragrapsus quadridentatus	170	96	17 °C	–	Ahsanullah and Arnott, 1978

exposure levels. Positive phototaxis and photokinesis are seen at 50 and 100 ppb Cu, but a decline in percent positive phototaxis relative to the control is evident (Fig. 2). At 150 ppb Cu, photoreversal to negative phototaxis at 10^{-3} to $10^{-4}\,Wm^{-2}$ occurs (Fig. 2). No photokinetic response is seen at 150 ppb Cu (Table 2).

At 15 ‰ salinity, the effects of 50 ppb Cu are more pronounced relative to those at 50 ppb Cu at 30 ‰. Photoreversal occurs (Fig. 3) and MLV is depressed (Table 3). In general, effects at 50 ppb Cu, 15 ‰ most closely resemble effects at 150 ppb Cu for 30 ‰, but a distinct photokinetic response is still present for nauplii at 15 ‰.

Discussion

The 24 h LC50 values for Stage II *B. improvisus* nauplii of 88 ppb Cu at 15 ‰ and >200 ppb at 30 ‰ represent generally intermediate values when compared to those for other marine crustacean zooplankton (Table 4). Barnacle nauplii exhibit neither exceptional sensitivity or resistance to Cu.

Within the 24 h test period, relatively increased toxic effects of Cu were observed at the lower test salinity. Similar salinity effects on Cu toxicity have been observed for isopods (Jones, 1975) and bivalves (Olson & Harrel, 1973), although salinity had no significant effect on Cu toxicity for lobsters (McLeese, 1974). *Balanus improvisus* normally occur in low salinity waters (Lang, 1979) and nauplii develop more rapidly in 15 ‰ relative to 30 ‰ laboratory cultures (unpublished data). Accordingly, increased toxicity of Cu at 15 ‰ probably does not reflect a lowering of test organisms resistance from suboptimal salinity stress (Vernberg *et al.*, 1973). These results may reflect the greater Cu

complexing capacity of the water as salinity increases (Schmidt, 1978a).

Temperature also appears to alter the acute toxicity of Cu; test species generally increase resistance to Cu with decreasing temperature (Eisler, 1977). At 30 ‰, *Balanus improvisus* nauplii exhibit a 24 h LC50 of about 165 ppb Cu at 26 °C (Lang *et al.*, 1979b) while Table 1 indicates that at 20 °C the value is greater than 200 ppb. However, these differences may not solely reflect a simple temperature effect, since variation in inherent Cu resistance in South Carolina (26 °C) and Rhode Island (20 °C) nauplii may also be a significant factor.

Stage II *B. improvisus* nauplii are active swimming larvae with a MLV of about 1.5 to 1.8 mm sec^{-1} at 20 °C when swimming under simulated dark conditions (light filtered at 830 nm) (Lang *et al.*, 1979a). Light-adapted nauplii exhibit a significant directional response to light intensities at about 10^{-6} Wm^{-2} (480 nm) while an increased MLV occurs above 10^{-3} Wm^{-2} (Lang *et al.*, 1979a). Similar behavior was observed for control groups in the present study. Control nauplii at 30 ‰ tend to have a slightly increased MLV relative to those at 15 ‰ (Table 2,3); salinity may indeed have a small effect on swimming speed (Lang *et al.*, 1980).

At 24 h, the MLV response of nauplii below photokinetic thresholds and exposed to 50 ppb Cu is significantly depressed at both 15 and 30 ‰. Phototaxis is also depressed or altered. Effects are more pronounced at 15 ‰, and is in agreement with salinity effects observed in the acute toxicity study. At 30 ‰, a gradient of progressively depressed MLV with increasing Cu concentration is evident (Table 2). The relative toxic effect of each test condition appears to be reflected by MLV response. For both salinities, the lowest Cu concentration that will suppress linear swimming speed is below 50 ppb Cu.

Additional studies (Lang *et al.*, 1979b) show that low concentrations of Cu (25 to 60 ppb Cu) can increase the MLV of *Balanus improvisus* relative to control responses. A complete MLV response curve for nauplii may involve an initial stimulatory effect at low Cu concentrations followed by a progressive decrease in MLV as lethal Cu levels are approached. Cu and other metals have been observed to induce transient stimulatory effects on various physiological parameters of marine animals (Stebbing, 1976). For the present study, we assume the senstivity of the nauplii and the exposure conditions resulted in a threshold for stimulatory effects below 50 ppb Cu while in the earlier study this threshold occurred between 60 ppb and 100 ppb Cu (Lang *et al.*, 1979b). Studies in progress should further clarify this response.

Stage II barnacle nauplii are normally strongly positively phototactic, but adverse conditions, such as extremes in salinity, temperature and light intensity or toxic chemicals are known to induce a photoreversal in naupliar response (Ewald, 1912). Directional responses for nauplii at 30 ‰ (Fig. 2) show a decline in percent positive phototaxis at 50 and 100 ppb Cu, with a reversal to negative phototaxis at 150 ppb Cu. Nauplii at 15 ‰ exhibit negative phototaxis at 50 ppb Cu (Fig. 3). Negative phototaxis is restricted to light intensities at or slightly below those inducing maximal positive response in control larvae.

Both MLV and DOT responses of barnacle nauplii are significantly altered by sublethal Cu concentration at 24 h. A gradient of response at 30 ‰ is evident, perhaps proportional to toxic effects of each test condition. MLV is first affected by Cu at lowest concentrations, followed by a decline in positive phototaxis, and finally, at highest concentrations, a reversal to negative phototaxis.

Assuming altered MLV and DOT represent a "limited specific response to a broad range of applied stressors" (Rosenthal and Alderdice, 1976), these parameters could be useful short-term, senstitive sublethal indicators of toxic effects. Further investigation of probable ecological significance of altered MLV-DOT is also needed if these parameters are to be employed for prediction of field and population effects (Miller, 1980).

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